PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
RCK-0017		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US04/37925	12 November 2004 (12.11.2004)	24 November 2003 (24.11.2003)

International Patent Classification (IPC) or national classification and IPC

IPC(8): C12N 5/00, 5/02, 15/00, 15/09, 15/63, 15/70, 15/74, 15/85, 15/87; A01K 67/00, 67/03, 67/027 and US Cl.: 435/325, 320.1, 455, 463; 800/13, 14

Applicant

THE ROCKEFELLER UNIVERSITY

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of ___ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of report with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
28 July 2005 (28.07.2005)	23 January 2006 (23.01.2006)
Name and mailing address of the IPEA/US Mail Stop PCT, Attn: IPEA/ US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	Authorized officer Thaian N. Ton Telephone No. (571) 272.1600

Form PCT/IPEA/409 (cover sheet)(July 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US04/37925

I. Basis of the report

1. With regard to the elements of the international application:*

☒ the international application as originally filed.

☒ the description:
pages 1-71 as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the claims:
pages 72-75, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the drawings:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the sequence listing part of the description:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item. These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. NONE

☒ the drawings, sheets/~~fig~~ NONE

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US04/37925

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims NONE	YES
	Claims 1-20	NO
Inventive Step (IS)	Claims NONE	YES
	Claims 1-20	NO
Industrial Applicability (IA)	Claims 1-20	YES
	Claims NONE	NO

2. CITATIONS AND EXPLANATIONS
Please See Continuation Sheet

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

V. 2. Citations and Explanations:
Claims 1-20 the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

Claims 1-6 lack novelty under PCT Article 33(2) as being anticipated by Trempus *et al.* The claims are directed to methods for isolating a self-renewing, multipotent cell by obtaining a cell from a sample and sorting the cells based upon the presence of CD34 and the amount of a selected slow-cycling cell marker expressed by the cell. The claims are also directed to cells isolated by the claimed method. Trempus teach the isolation of epithelial cells with stem and progenitor cell characteristics using a CD34 specific antibody, and identifying in that population a subset of cells also expression alpha-6 integrin. See Abstract. Particularly, they teach that keratinocytes were isolated from the dorsal skin of mice, cells were separated by flow cytometry and the resulting cells isolated. See Materials and Methods, pp. 502-503. Thus, Trempus teach the claimed invention because they teach a progenitor cell isolated by the presence of both CD34 and another marker expressed by the cell.

Claims 7, 9-16 lack novelty under PCT Article 33(2) as being anticipated by Yuan *et al.*, or Roy *et al.*, or Fujikawa *et al.* or Coffin *et al.* Note that claims 9-16 are directed to cell populations, produced by a particular method. The method by which the cells are produced fails to differentiate the cells from the art, thus, art that teaches the products teaches the claims.

Yuan teach the generation of a transgenic mouse expressing EGFP under the CNP promoter. They observe the expression of EGFP, and isolated oligodendrocyte progenitor cells from the mice using fluorescence activated cell-sorting (FACS). See Methods and Materials, p. 530-531.

Roy teach the identification isolation of oligodendrocyte progenitor cells from adult human subcortical white matter. Particularly, they teach the dissociation and culture of cells from adult human brain (p. 9987, Materials and Methods, 2nd column), the transfection of these cells with a transgene eoncding the CNP2 promoter with targeted GFP expression. They teach that the cells expressing GFP were then sorted using flow cytometry and a FACS machine. See p. 9989, 1st column.

Fujikawa teach the purification of isolated hepatic progenitor cells using GFP-transgenic mice, and isolating cells from the mice. Paritcularly, they teach that GFP-transgenic mice, which express GFP under the cytomegalovirus enhancer-beta-actin promoter. Liver tissues were isolated from the mice, and then the cells were sorted and characterized. The cells were then sorted by FACS and analyzed. See pp. 163-164. Fujikawa teach that the cells that were sorted had immature characteristics (p. 166, 2nd column) and that the cells showed *in vitro* differentiation potential to produce hepatocytes. See p. 167, #3.5.

Coffin teach the generation of populations of transduced human primary cells by FACS sorting using GFP expression.

Supplemental Box

Particularly, they teach that human hematopoietic stem cells were transduced using a HSV1 vector expressing GFP. See Abstract. The transduced cells were then sorted to remove GFP-negative cells.

Claims 7, 8-16 lack novelty under PCT Article 33(2) as being anticipated by Bartz *et al.* Bartz teach the isolation of immature dendritic cells from Langerhans cells by sorting using two markers, CD34+ or CD133+ (see p. 139, #2.3) and then cells from this population were further sorted and isolated using CLA expression (p. 139, #2.4). The resulting cells were the isolated and cultured and then analyzed (p. 139, #2.5).

Claims 17-18 lack novelty under PCT Article 33(2) as being anticipated by Punzel *et al.* Puzel teach the culture and expansion of human hematopoietic stem cells, by growing the cells on fibroblast feeder cells using LTBMC medium. See p. 93, 2nd column. Note that the LTBMC medium that they teach contains IMDM, which contains calcium chloride (.219 g/L). Thus, they anticipate the claims.

Claims 19-20 lack novelty under PCT Article 33(2) as being anticipated by Krestel *et al.* Krestel teach the generation of transgenic mice using a transgene encoding humanized GFP that is regulated by doxycycline. Expression was activated when the transcription factor tTA (tet-dependent transcirpiton activator) was expressed by the transgene. See Abstract and Materials and Methods.